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Isolation and Characterization of New Mutations in the mig-10 gene of Caenorhabditis elegans

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Isolation and Characterization of New Mutations in the *mig-10* gene of *Caenorhabditis elegans*

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Abstract

During development in *C.elegans*, several neurons migrate to specific locations within the body; this is important in the formation of the nervous system. Mutations in the *mig-10* gene have been phenotypically identified by incomplete migration of those cells and the truncation of the excretory cell. This project focuses on identification of additional mutations in the gene, isolated by non-complementation screening, and characterized using PCR and restriction digestion. Missense mutations may identify functional domains in the MIG-10 protein.

Authorship

Acknowledgements

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1 Introduction

1.1 The mig-10 gene functions in neuronal migration and axon outgrowth

During early embryonic development, several neurons are moving throughout the embryo to specific locations to carry out specialized functions. This neural migration is a carefully regulated process to ensure that specific neurons move to the location where they are meant to function. When the genes regulating neural migration are mutated it can disrupt the migration of those cells which affects their ability to function properly. This project will attempt to characterize novel mutations in the *mig-10* gene, a gene responsible for neural migration in the model organism *C. elegans*.

The *mig-10* gene in the nematode *Caenorhabditis elegans* (*C. elegans*) plays a role in the regulation of migration of several embryonic neurons; specifically the Canal Associated neurons (CAN), Anterior Lateral Microtubule cells (ALM), and Hermaphrodite Specific neurons (HSN). CAN and ALM migrate from the anterior towards the posterior, stopping at approximately half the length of the worm, and HSN migrate from the posterior to the anterior to a similar position (*Manser and Wood, 1990*).

The *mig-10* gene is also essential in the process of axon guidance and the development of the excretory canal. Axon guidance is a part of neural development in which specific axons navigate along specific pathways to reach their final destination or target (*Lundquist, 2003*). The extended end of a developing axon is a special structure called a growth cone *(*Figure 1*)* which contains finger-like filopodia, exploring and detecting the changes in its extracellular environment. The excretory cell in *C. elegans* is located on the

ventral side near the developing pharynx *(*Figure 2*).* A complicated network of tubular epithelia is formed from a single excretory cell. This tubular formation is a fascinating example of cell morphogenesis which is mediated through interactions of specific receptors on the membrane with the extracellular environment. Canal tips might be stimulated and guided during the development of excretory canal by utilizing the same cues and mechanism as do the neural migration and outgrowth (*Buechner, 2002*).

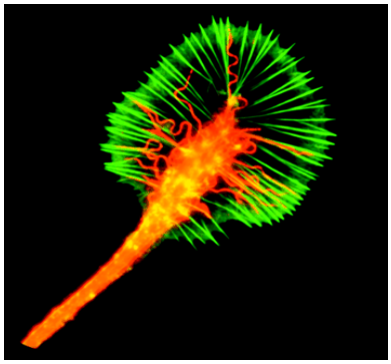

Figure 1: Structures of a Neural Growth Cone involved in Axon Guidance
Picture taken from The Journal of Cell Biology, 2002, 157 (5)

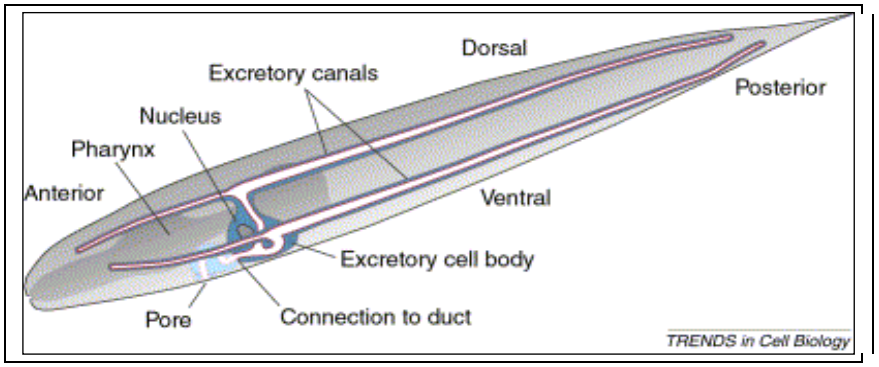

Figure 2: Structure of the excretory canals in a late larval stage worm
The position of the excretory cell body is shown beneath a grey shadow representing the pharynx. Picture from Buechner, 2002

It is hypothesized that several neurons migrate to their final destination through a series of attractive and repulsive guidance cues by four major conserved families of axon

guidance molecule: netrins, slits, semaphorins and ephrins *(Yu and Bargmann, 2002).* Axon outgrowth and the development of excretory cells are also guided by these extracellular guidance molecules. The growth cones at the end of an axon contain receptors that recognize specific guidance cues. Axons locate their targets by detecting chemical attractants or repellants located either on the surface of guide cells or in the extracellular matrix. These cues enable the axons either to move toward their targets or away from certain targets respectively. Once a growth cone senses a guidance cue, the receptors in the growth cone send a signal to tell the axon the correct orientation for migration. The growth cones can also respond to different guidance molecules at different stages during their development and they can change direction accordingly. Some guidance molecules are acting to attract neurons and others are repelling neurons, depending on the context of axon guidance and the neural migration. For example, guidance molecules netrin and slit can function either as attractants or repellents to direct axons to their final targets (*Yu and Bargmann, 2001*).

MIG-10 has been identified as playing a role in both the pathways of slit-dependent and netrin-dependent axon guidance. The MIG-10 protein is a cytoplasmic adaptor protein that functions downstream of these cues, specifically UNC-6/Netrin and SLT-1/Slit (*Manser et al, 1997; Quinn et al, 2006*). A mutated *mig-10* gene results in the phenotype of incomplete migration of CAN, ALM, and HSN, as well as the phenotype of a truncated excretory canal (*Maner and Wood, 1990*). Furthermore, over-expression of MIG-10 in the absence of both guidance cues UNC-6 and SLT-1 cause neurons such as AVM and PVM to migrate to different orientations or directions, displaying a multipolar phenotype. When MIG-10 is over-expressed in the presence of either UNC-6 or SLT-1, the multipolar phenotype is suppressed and a monopolar phenotype is expressed. Together, these studies

suggest that MIG-10 alone promotes the activity of axon outgrowth without a directional response, however, in the presence of guidance cues such as UNC-6 or SLT-1 the outgrowth activity is guided in a directional path and this directional guidance response is enhanced by MIG-10 over-expression (*Quinn et al, 2006*).

MIG-10 has a similar function to UNC-34 as they act in overlapping pathways. UNC-34 is a member of the Eva/VASP protein family, which is involved in actin polymerization and cellular mobility. This is demonstrated because animals with mutations in both *mig-10* and *unc-34* are generally lethal and those worms that do not die are found to have severe axon guidance defects (Quinn et al, 2006). Both of these genes affect filopodia formation, which is needed for axon growth and guidance. UNC-34 is essential for formation of filopodia downstream of netrin; however the guidance can be accomplished by alternate motility mechanisms, which are hypothesized to be stimulated by MIG-10 and Rac pathways (*Chang et al, 2006*).

1.2 Known mig-10 Mutations

A single base pair change (C→T) within exon 3 of the *mig-10* gene, the *mig-10 (ct41)* allele, results in a premature stop codon. This mutation produces a truncated and nonfunctional MIG-10 protein (*Manser and Wood, 1990*). A mutation in the *mig-10 (e2527)* allele occurs at the splice acceptor site (TTTCAG→TTTCAC). The phenotype for this mutation is similar to that of the *mig-10 (ct41)* allele, it causes incomplete migration; however *e2527* is regarded as a weaker phenotype because only ALM is affected with high penetrance whereas all three sets of neurons are affected with high penetrance in *ct41* (*Manser et al, 1997*).

The effects of the *ct41* mutant allele suggest that the *mig-10* mutation is a defect in the mechanism of migration because of the incomplete migration of all three major migratory neurons: CAN, HSN, and ALM. This occurs despite the fact that CAN and ALM migrate from anterior to posterior and HSN migrates from the posterior towards the anterior.

1.3 Structure and Interactions of MRL proteins

Although not identical, Mig-10 shares similarities with mammalian SH2 domain proteins, especially Grb7 and Grb10 *(*Figure 3*).* While Mig-10 does not specifically contain a SH2 domain, they share a pleckstrin homologous domain (PH domain), proline-rich regions (PR domain) and Ras-associated domain (RA). The Mig-10 protein is also homologous to the vertebrate RIAM, and lamellopodin (Lpd). The RIAM-related adaptor molecules are named the "MRL family (Mig10/RIAM/Lpd). The conserved domains shared by the MRL family are commonly found in signal transduction pathways *(Krause et al, 2004; Lafuente et al, 2004).*

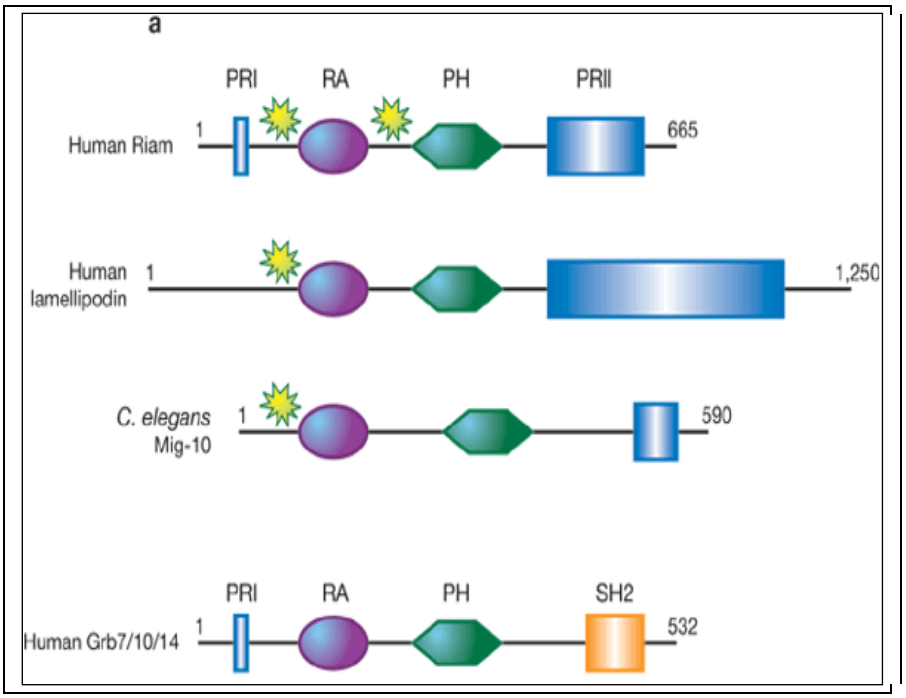

Figure 3: MIG-10, RIAM, Laellipodian and Human Grb7, Grb10, and Grb14 adaptor proteins share a conserved domain structure
PR1 and PR2, proline-rich domains; RA, Ras-association domain; PH, pleckstrin-homology domain; SH2, Src-homology-2 domain; yellow star, putative coiled-coil domain. Picture taken from Legg et al, 2004.

The MRL family of proteins is found to interact with Ena/VASP proteins. The Ena/VASP proteins are a conserved family of regulatory proteins which play an important role for the actin-based motility, fibroblast migration and axon guidance. Ena/VASP is implicated in actin filament elongation by shielding the end of the filament from the capping proteins. The Ena/VASP protein has an amino-terminal EVH1 domain, a carboxy-terminal EVH2 domain and a proline-rich central region. The EVH2 domain is involved in elongation of actin filaments while the EVH1 domain likely binds to the proline rich region of MRL protein (*Krause et al, 2004*).

Further understanding of the function of MIG-10 will come from study of the PH region or the RA region from its homologs. These domains function in protein-protein interactions, binding of small molecules, or localization of proteins to membranes. The PH

region has functions in signal transduction or in the cytoskeleton. Structural analysis of the PH region from several different proteins has revealed a common fold consisting of two antiparallel β sheets and a long C-terminal α helix *(Manser et al, 1997).* It is proposed that the PH domain in Lamellipodin binds to phosphatidylinositol (3, 4) bisphosphate (PtdIns (3, 4) P2). This phosphoinositide is located both at the plasma membrane and at the nuclear membrane. Therefore the localization of PH domain to the plasma membrane is crucial to trigger the Ena/VASP localization at the plasma membrane (*Krause et al, 2004*). By contrast, researchers have not yet found any phosphoinositide that binds to the PH domain of RIAM. On the other hand, the RA domain of RIAM is found to be associated with Rap1, a small GTPase which is closely related to Ras. The Rap1 has several implications in cell adhesion and it is activated through numerous receptors, including receptor tyrosine kinases and cytokine receptors (*Laufente et al, 2004*).

1.4 A Model for the Mechanism of MIG-10

A model is proposed to study the function of MIG-10 in response to some specific guidance cues, such as UNC-6/Nectrin and SLT-1/Slits (Figure 4). When the receptor in a specific axon that detects the extracellular guidance molecule is activated, it promotes the activation of Ras-related GTPases and phosphoinositide 3-Kinase (PI3K). PI3K catalyzes the production of PI(3,4)P2 from PI(4)P. Afterward the MIG-10 protein localizes to the vicinity of cell membrane and associates with the Ras-related protein and PIP2 via its RA domain and its PH domain respectively. The localization of Ena/VASP protein such as UNC-34 is achieved by the interaction of its EVH1 domain with the PR domain of the MIG-10 protein. Afterward, actin polymerization occurs in response to the activation of UNC-34 (*Quinn et al, 2006; Chang et al, 2006*).

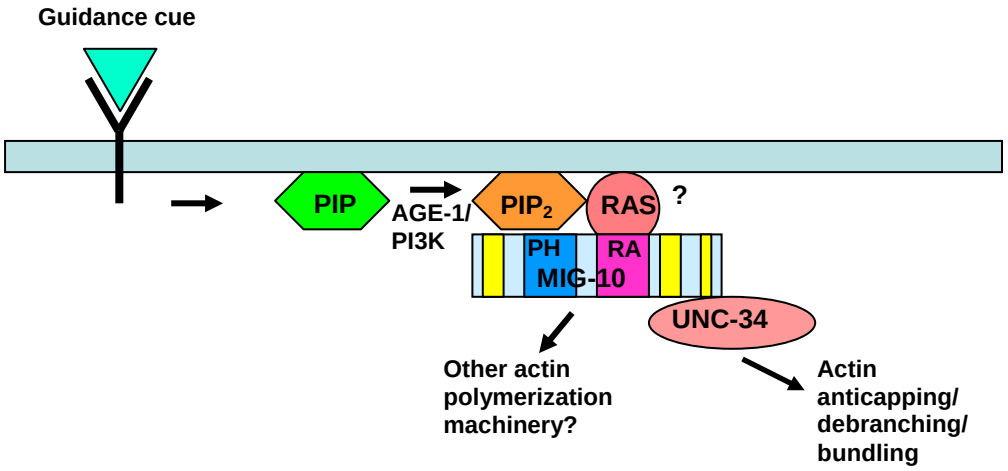

Figure 4: A Model for the Mechanism of MIG-10
Picture taken from Ficociello and Ryder, unpublished

1.5 C. elegans: Model Organism

C. elegans is used as a model organism for a variety of reasons. Worms respond to a diverse range of stimuli from their environment, including touch, smell, taste, and temperature. However *C. elegans* have a much simpler nervous system than the nervous system of *Homo sapiens*, with an adult worm having only 302 neurons compared to approximately a hundred billion neurons in humans, which makes *C. elegans* ideal to study.

Another reason to study *C. elegans* is that they also have a relatively small genome. It is estimated that the human genome consists of 3 billion base pairs, compared to *C. elegans* consisting of only about 97 million base pairs divided into 6 chromosomes *(Hodgkin, J., 2005).* Their small genome has also been completely sequenced. This means that any mutations can be identified in the genetic code. Their genome is also easy to

manipulate in a laboratory. These factors combined with the short life cycle of *C. elegans* enables the easy study of specific genes.

C. elegans are used to study neural migration and development because of several key features. Their systems are similar enough to higher organisms that the understanding gained from the study of the *mig-10* gene of *C. elegans* can be applied to more complex organisms such as humans. For example the MIG-10 protein contains the Ras association domain, a PH (pleckstrin homology) domain and a proline-rich motif. These traits and genes are homologous to vertebrate cytoplasmic adaptor proteins, such as RIAM, lamellopodin and GRB7 (*Quinn et al, 2006*). It is also possible to study how a change in the organism affects development because the cell divisions and movement that occur during development have been described and characterized and there is limited to no variation amongst the wild-type worms (*Manser and Wood, 1990*).

1.6 Genetic Screens

Genetic screens are used in order to isolate specific mutations in the genome, especially with *mig-10* mutations in *C. elegans* because mutations in this gene produce a reasonably clear phenotype that can be observed under a fluorescent microscope and can therefore be easily isolated. There are several types of screens that can be used. One such method is non-complementation screen which utilizes a mutagen, ethane methyl sulfonate (EMS), to induce a point mutation in the sperm and oocytes of wild-type hermaphrodites.

The non-complementation begins with a heterozygous strain for a recessive mutation in the gene of interest. This screen looks at the progeny of the F1 generation for the desired phenotype and then at the F2 progeny of these worms to get the homozygous alleles of new mutation. The advantage of the non-complementation screen is that the

phenotype will only result from new mutations in the desired gene. Non-complementation occurs when two mutations, in the same gene, but on opposite chromosomes, result in a mutant phenotype. If a new mutation is produced it would fail to complement the old mutation present on the other chromosome, resulting in the Mig phenotype. Another possible screen is the simple screen, which also utilizes a mutagen such as EMS. Unlike the non-complementation screen, simple screens only examine the F2 generation, for the desired phenotype. However there are typically multiple genes in which a mutation can produce the desired phenotype. In order to determine if the phenotype is the result of a new mutation in the gene of interest the initial step is to perform non-complementation with an animal homozygous for the old *mig-10* mutation to confirm that the mutation is on the same gene. The results of the screen can then go through the process of Polymerase Chain Reaction and gel electrophoresis.

1.7 Project Objectives

The objective of this Major Qualifying Project (MQP) was to identify novel missense mutations in the *C. elegans mig-10* gene, excluding null mutations. The presence of a new mutation was indicated by several possible phenotypes: a truncated Excretory Cell, or partial to lack of migration by the CAN, HSN or ALM cells. Identifying such mutations would help us to identify additional functional domains of the MIG-10 protein which likely interact with several downstream effectors in the signal transduction pathway *(*Figure 4*).*

The project followed a simple process to identify and characterize new mutations. First, the appropriate genetic screen for identifying new *mig-10* mutations was chosen. This screen then was used to attempt to identify new mutations in the *mig-10* gene. Putatives

with new mutations were characterized by PCR, restriction digestion and gel electrophoresis. If a new mutation was isolated, it would be sequenced, and functional domains identified if possible.

The screen used for this project was a non-complementation genetic screen and the worm strains used in this screen were heterozygous for the *mig-10* gene. The mutagenized parental strain gave rise to less than one percent of progeny carrying a new mutation. The putative with a new mutation that failed to complement the old mutation would be singled out on a plate. Afterward the new mutation was homozygosed in the F2 generation. Several additional genes were incorporated into the parental strain (*dpy-17* and *unc-32*) and were used to distinguish worms with new mutations from old one in F2 generation.

The ideal novel mutation would be a missense mutation in which a single base pair was changed to cause substitution of an amino acid with different properties. The change of a single base pair would make it possible to see which section of the gene has an effect on the functionality of the protein, thus allowing for more focused study on that area of the gene and protein.

2 Methods and Materials

2.1 Nematode Growth Medium (NGM) Agar Plates

Worms were cultured on NGM plates with a spot of *E.coli*. To make these plates 1L of NGM media (51.3mM Sodium Chloride, 1.7% agar, 0.25% Peptone) was autoclaved for 20 minutes. Additional constituents were added using sterile technique to a final concentration of 1mM Calcium Chloride, 1.3mM Cholesterol, 1mM Magnesium sulfate and 25mM Potassium Phosphate buffer (pH6.0). The medium was dispensed to each plate sterilely and was left to dry under a fume hood. An overnight culture of OP50 *E.coli* was made from a single colony in LB Broth. Once the plates were dry, a spot of the culture was placed in the center of each plate using a sterile Pasteur pipet.

2.2 Maintained Strains

Parental and comparison strains were maintained throughout the project for strains RY0180 and RY0181. The parental strains were used as stocks and were maintained in a heterozygous state displaying the wild-type phenotype. The comparison strains were used to compare phenotypes to worms being screened and were maintained in a homozygous state. Comparison strains were maintained for the Dpy, Mig phenotype, the Dpy, Unc-32 phenotype and the Unc-36, Mig phenotype as these were the hardest to identify while screening. Dpy worms are dumpy; they are shorter, fatter and football shaped. Unc-32 worms tend to coil or not move as easily, Unc-36 worms adopt unnatural postures and are slower to move and respond. To maintain heterozygous strains one worm in the L4 stage with the wild type phenotype was singled to three new plates once or twice a week. To

maintain homozygous strains three worms in the L4 stage with the desired phenotype were transferred to new plates once or twice a week.

Table 1: Genotype of Maintained Strains

Strain Name	Parental Strain	Comparison Strain(s)	
RY#0180	$\frac{+\ \text{lon-1}\ +\ \text{unc-32}}{\text{dpy-17}\ +\ \text{mig-10}\ +}$; bgIs312	$\frac{\text{dpy-17}\ +\ \text{mig-10}\ +}{\text{dpy-17}\ +\ \text{mig-10}\ +}$; bgIs312	
RY#0181	$\frac{\text{dpy-17}\ +\ +\ \text{unc-32}}{+\ \text{unc-36 mig-10}\ +}$; bgIs312	$\frac{\text{dpy-17}\ +\ +\ \text{unc-32}}{\text{dpy-17}\ +\ +\ \text{unc-32}}$; bgIs312	$\frac{+\ \text{unc-36 mig-10}\ +}{+\ \text{unc-36 mig-10}\ +}$; bgIs312

A bgIs312 transgene was used to express GFP in the worms. The GFP labels the excretory canal and allows the *mig-10* phenotype to be easily observed under a fluorescent dissecting microscope. Under fluorescent light the canal appears truncated in worms with the *mig-10* phenotype compared to wild-type worms.

2.3 EMS Mutagenesis

Three days before mutagenesis three L4 stage of either RY0180 strain or RY0181 strain were picked to each of 5-10 plates to generate a significant number of L4 stage worms. The mutagenesis was started three days later with the worms being washed off all 5-10 plates into a 15mL tube with M9 solution (22mM KH_2PO_4, 34mMNa_2HPO_4, 85.4mM NaCl, and 1mM $MgSO_4$). The tube was centrifuged at 200xg for two minutes, and the supernatant was removed without disturbing the worm pellet. Then the pellet was suspended in 10mL M9. This process was repeated two more times. After the last wash, the worms were resuspended in 4ml M9 containing 20ul EMS (ethane methyl sulfonate, Sigma #M-0808) under the hood. Pipette tips that touched EMS were discarded in a 50mL conical tube filled to the 5mL mark with the NaOH pellets. The tube containing the worms was gently rotated on the nutator for 4 hours to complete the mutagenesis. After mutagenesis of

the P0 generation worms was complete the worm tube was centrifuged at 200xg for 2 minutes and washed with 10mL M9 solution. This step was repeated four times. After the last centrifugation, supernatant was removed and the worms were resuspended in a small amount of M9 and then transferred to the edge of the bacterial lawn on new NGM agar plates using a Pasteur pipette. The plates were dried at room temperature for at least one hour. Three to five L4 or young adults of the P0 generation were picked to 10-20 new plates which were incubated at 20ºC overnight. Lastly, the worms from each plate were transferred to a corresponding new plate the day after mutagenesis as well as two days after mutagenesis.

2.4 10 Worm PCR

Lysis Step

2.5uL lysis buffer and enzyme (50mM KCl, 10mM Tris, pH 8.2, 2.5 mM MgCl2, 0.45% NP-40, 0.45% Tween 20, 0.01% DNA free gelatin and 0.12mg/mL Proteinase K) were placed into the cap of each PCR tube. At least ten worms were picked into each tube cap. Then the tubes were microcentrifuged for 5 seconds before adding 50uL mineral oil to overlay the pellet. The tubes were kept in a -80ºC freezer for at least 30 minutes. Afterward the worm tubes were placed into the thermocycler to undergo the lysis for 1 hour at 65ºC and inactivation of Proteinase K for 15 min at 95ºC.

PCR Amplification

The primers were mig-10 WT1 (5' TGTTTGAAT TTTCAGAAT CCGC 3') and *mig-10* WT2 (5'TGTTTCTTCTCACAATCCAACC 3'). The reaction mixture (25uL) contained 1X Long Template PCR System Buffer 3 (Roche Brand, Catalog #1742663), 0.25mM

dNTP, 0.3uM Primer *mig-10* WT1, 0.3uM Primer *mig-10* WT2 and 3.75U of Taq Polymerase (New England BioLabs, Catalog #M267L), and 2.5uL template DNA preparation from lysis step. The short PCR amplification began with initial denaturing at 94°C for 10 min, then the amplification was performed for 30 cycles, consisting of denaturing at 94°C for 30 s, annealing at 60°C for 1 min, and primer extension at 72°C for 2 min, with a final extension at 72°C for 10 min.

2.5 Restriction Enzyme Digest

10uL DNA amplified products from each tube were digested with 5U Hpy188I endonuclease (New England BioLabs, Catalog #R0617L) in a reaction mixture (15ul) containing dH20 and 1X NEBuffer 4 (New England BioLabs, Catalog #B7004). The 1X NEBuffer 4 consists of 20mM Tris-acetate, 10mM potassium acetate, 10mM Magnesium Acetate and 1mM dithiothreitol. Each tube with digestion reaction was incubated at 37°C overnight.

2.6 Gel Electrophoresis

The samples were electrophoresed in 1% or 2% agarose gel with 1X TBE (Tris-borate-EDTA) buffer. The 100bp DNA Ladder (New England BioLabs, Catalog #N3231L) was used as a marker in this gel electrophoresis. The gel was run at 110-130 Volts for 2 hours.

3 Results

In order to identify new missense mutations in the *mig-10* gene, we performed a non-complementation genetic screen. Putative mutants were characterized using PCR and gel electrophoresis.

3.1 Phenotype of Genetic Markers used in Screens

The phenotypes of *C. elegans* such as *dpy-17 (*Figure 5*e)* and *unc-36 (*Figure 5*c)* could be observed under a dissection microscope. Worms with a mutation in the *dpy-17* gene appeared short, fat and football shaped. Worms with a mutation in the *unc-36* gene moved slowly and adopted unnatural positions compared to wild-type worms.

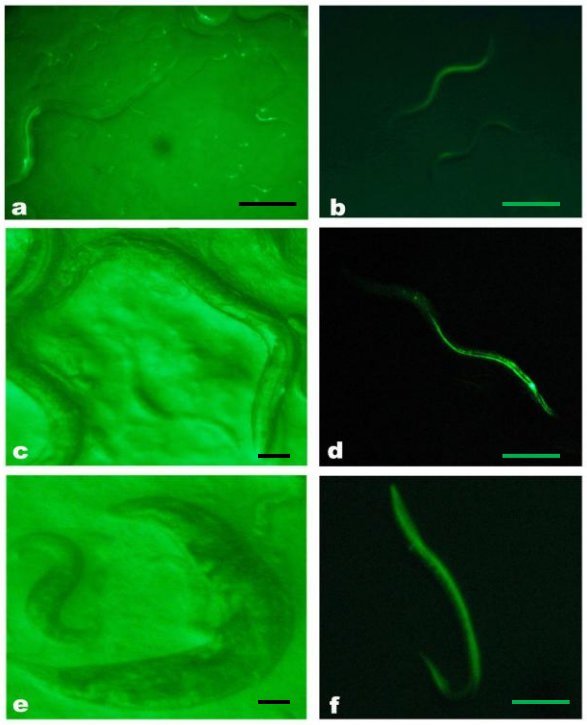

Figure 5: Micrographs of *C. elegans* showing Different Phenotypes
(a) *Mig phenotype;* ***(c)*** *Unc-36 phenotype;* ***(e)*** *Dpy-17 phenotype;* ***(b), (d)*** *and* ***(f)*** *Wild Type phenotype. All strains shown in Figure 5 contained the bgIs312 transgene, which was used as an indicator for mig-10 phenotype of truncated excretory canal. Scale bar: 50um*

However, the phenotypes of the *mig-10* mutation such as migration defects in ALM, HSN and CAN neurons, the length of the excretory canal, withered tail and egg-laying defects were not easily observed under a dissecting microscope. A bgIs312 transgene or array was incorporated in the mutagenized strain and used as an indicator for the Mig-10 phenotype of a truncated excretory canal. The micrographs of strain RY0181 heterozygous worms *(*Figure 5*b, d, f)* were taken under fluorescent light and show the full length excretory canal. The Mig-10 phenotype showing the truncated excretory canal can be seen in Figure 5a.

3.2 Non-complementation Genetic Screen of Strain RY0180

The first screen used strain RY0180 and about 1600 haploid genomes were screened. The mutagenized P0s were expected to produce 50% worms with wild type phenotype, 25% worms with the Dpy, Mig phenotype and 25% worms with the Lon, Unc phenotype among F1 progeny *(***Figure 6***).* Rare new alleles of *mig-10* gene were distinguished by their Mig phenotype. Worms with the Mig phenotype only were picked and singled out to new plates so their progeny could be examined. The new mutation of the *mig-10* gene would result in a Lon, Unc, Mig phenotype *(***Figure 6***).* Worms with Lon, Unc, Mig phenotype were isolated from the F2 progeny and their DNA was extracted for PCR analysis.

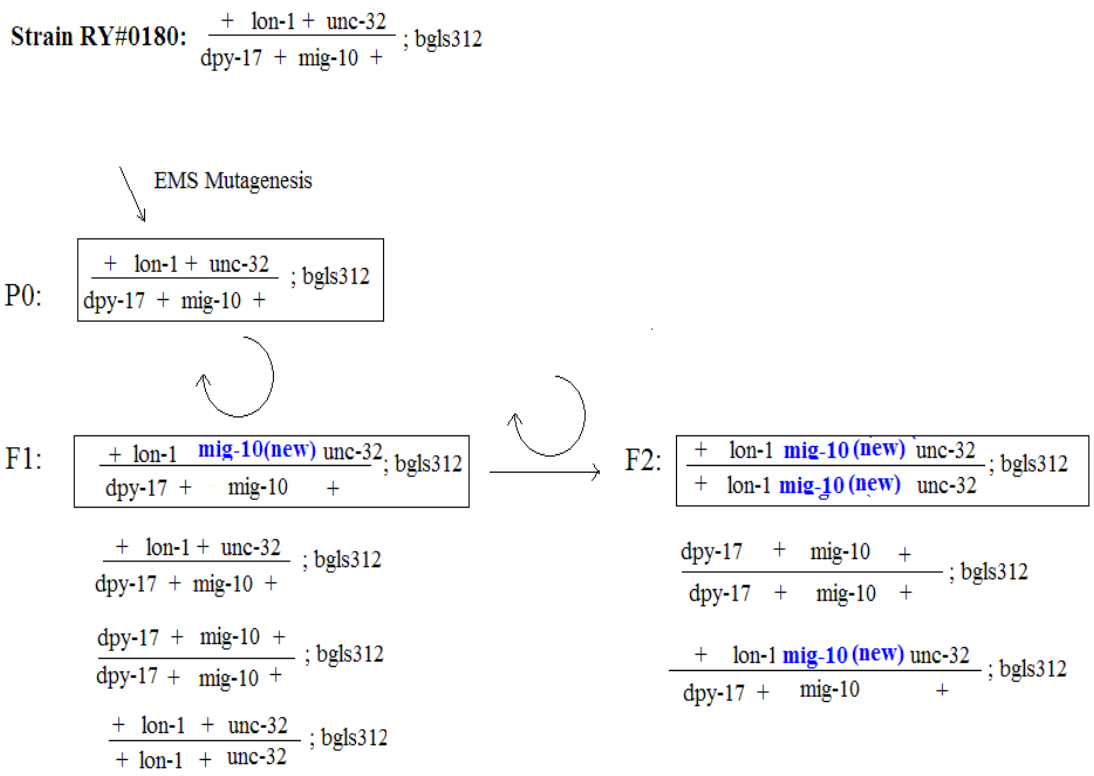

Figure 6: Screening Process for Strain RY0180

Crossing over in strain RY0180 could produce a Mig phenotype in the F1 generation, but it would not produce the Lon Unc Mig phenotype in the F2 progeny *(*Figure 7*)*. This is able to occur because *dpy-17* is 2.4 map units away from *mig-10*, which means that 2.4% of the gametes will show crossing over between the two markers. If there was a crossover between *lon-1* and *mig-10* or *dpy-17* and *lon-1* among the mutagenized P0 generation, worms with a *lon-1 mig-10* or *mig-10* chromosome could arise and would have the Mig phenotype if it was paired with the *dpy-17 mig-10* chromosome of the parental strain. However the progeny of these worms would not have the Lon Unc Mig phenotype that indicated a new allele *(*Figure 7*)*. A crossover between *mig-10* and *unc-32* would not result in a false positive Mig phenotype.

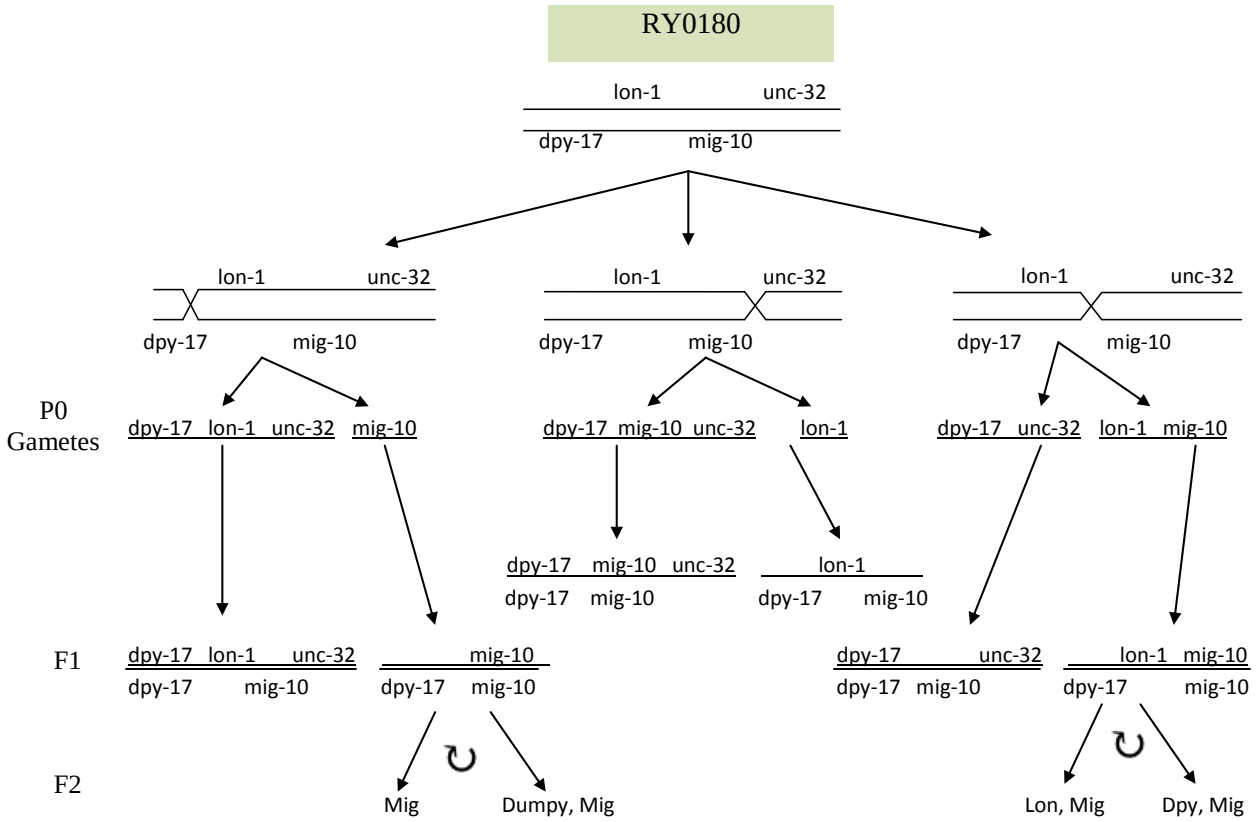

Figure 7: Possible Crossing over Events in Strain RY0180.
There are three possible places that crossing over can occur in this strain resulting in 6 possible gametes that would not occur in the strain normally. When these gametes are paired with the dpy-17 mig-10 chromosome of the original strain some of the resulting phenotypes could be deceptively like the putative phenotypes in F1 generation but will not result in the Lon, Unc, Mig phenotype that denotes new mutations in F2 generation.

3.3 Wild-type Mig-10 gene vs. Known mig-10 (ct41) Mutation

The wild-type *mig-10* gene has four Hpy188I restriction sites, and a known mutation (ct41) in the *mig-10* gene knocked out one of the four restriction sites at 378bp *(*Figure 8*)*. The novel mutation would not have the same restriction map as the known mutation because the novel missense mutation would be likely to occur elsewhere on the chromosome than the restriction site at 378bp. If the novel missense mutation knocks out one of the four restriction sites other than at 378bp, it would create a different restriction map from known *mig-10* mutation. If the novel missense mutation did not occur at any of

the four restriction site, then the restriction map of novel mutation would resemble the restriction map of the wild-type *mig-10* gene.

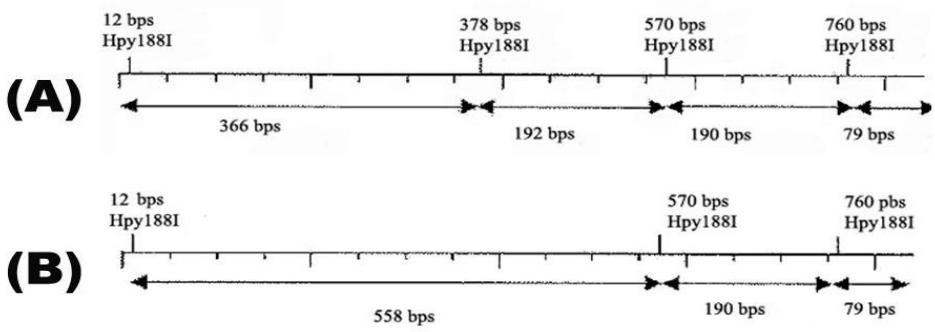

Figure 8: Restriction Map of (A) Wild Type *Mig-10 gene* and (B) Known *mig-10(ct41)* mutation
Pictures taken from Glover, R and Morin, S MQP project.

3.4 Characterization of LUM worms from Strain RY0181

Four putatives from the genetic screen of Strain RY0181were suspected to include novel missense mutations of the *mig-10* gene because their progeny appeared to have the Lon, Unc, Mig phenotype (LUM phenotype). At least ten worms with the LUM phenotype from each putative plate were picked to undergo the lysis reaction. Each sample was used to run a PCR reaction using primers specific to the *mig-10* gene. This was followed by a digestion reaction with the Hpy188I restriction enzyme and gel electrophoresis. The digested samples had similar sized DNA fragments to the DNA fragments of the known *mig-10(ct41)* mutation (Figure 9). Therefore no new mutation had been generated in screen 1 by using Strain RY0180. These results suggest that the phenotypes thought to be LUM phenotype were actually the Lon, Mig crossing over result seen in Figure 7. The Lon, Mig worms also display the "withered tail" phenotype and its effects are amplified because of the length of the worm. This makes the worm look as though it carries a mutation in the

unc-32 gene but it is instead uncoordinated due to a separate phenotype resulted from the *mig-10* gene.

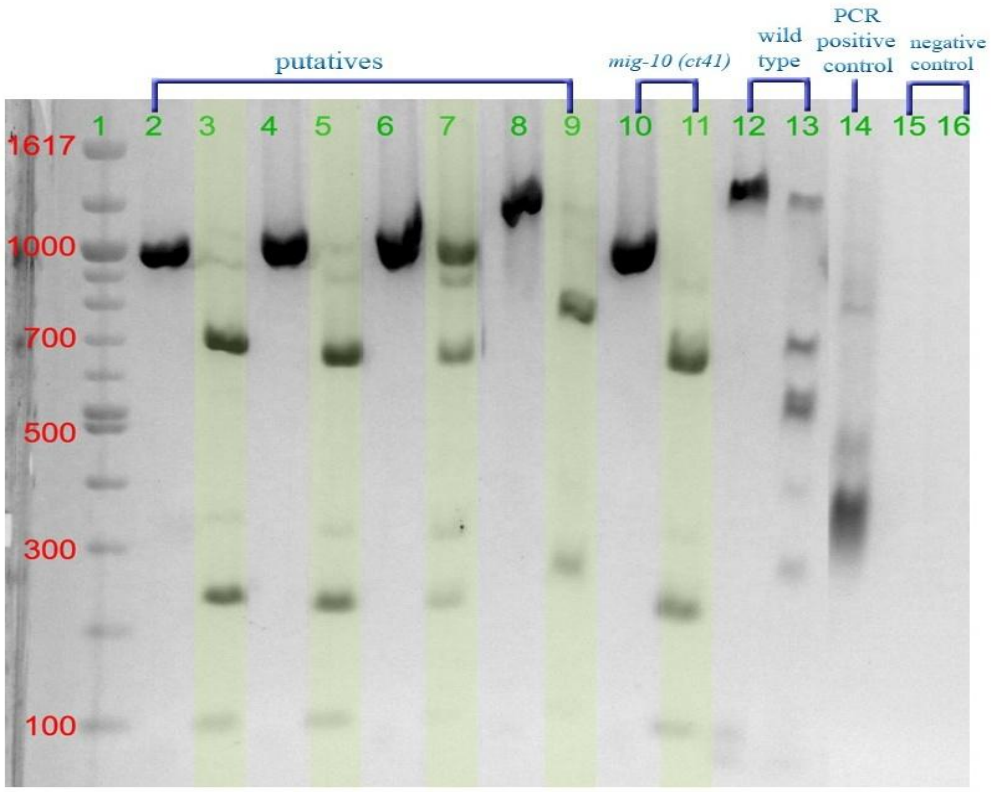

Figure 9: The Gel Results of Digested and Undigested Samples
From Left to Right: Lane 1: marker; Lane 2, 4, 6 and 8: undigested sample of four putatives (L8, L10, S10 and Z5) having LUM phenotype; Lane 3, 5, 7 and 9: digested sample of four putatives having LUM phenotype; Lane 10-11: undigested and digested mig-10 (ct41) mutation; Lane 12-13: undigested and digested wild-type mig-10; Lane 14: PCR positive control; Lane 15-16: undigested and digested negative control (containing no DNA).

3.5 Non-complementation Genetic Screen of Strain RY0181

Due to the phenotypes that resulted from the non-complementation screen of strain RY0180 we concluded that crossing over was happening often. In order to avoid crossing over during the genetic screen, we used a different strain, RY0181, to make the genetic screen more approachable. The same strategy was used for Strain RY0181 as for Strain RY0180. There were different types of uncoordinated movements: *unc-32* gave rise to

worms with coiled movement and *unc-36* made worms move slowly and adopt unnatural positions. Strain RY0181 hermaphrodites with the wild-type phenotype gave rise to P0 progeny with three different phenotypes: 50% wild-type worms, 25% worms with Dpy, Unc-32 phenotype and 25% worms with Mig, Unc-36 phenotype *(Figure 10).*

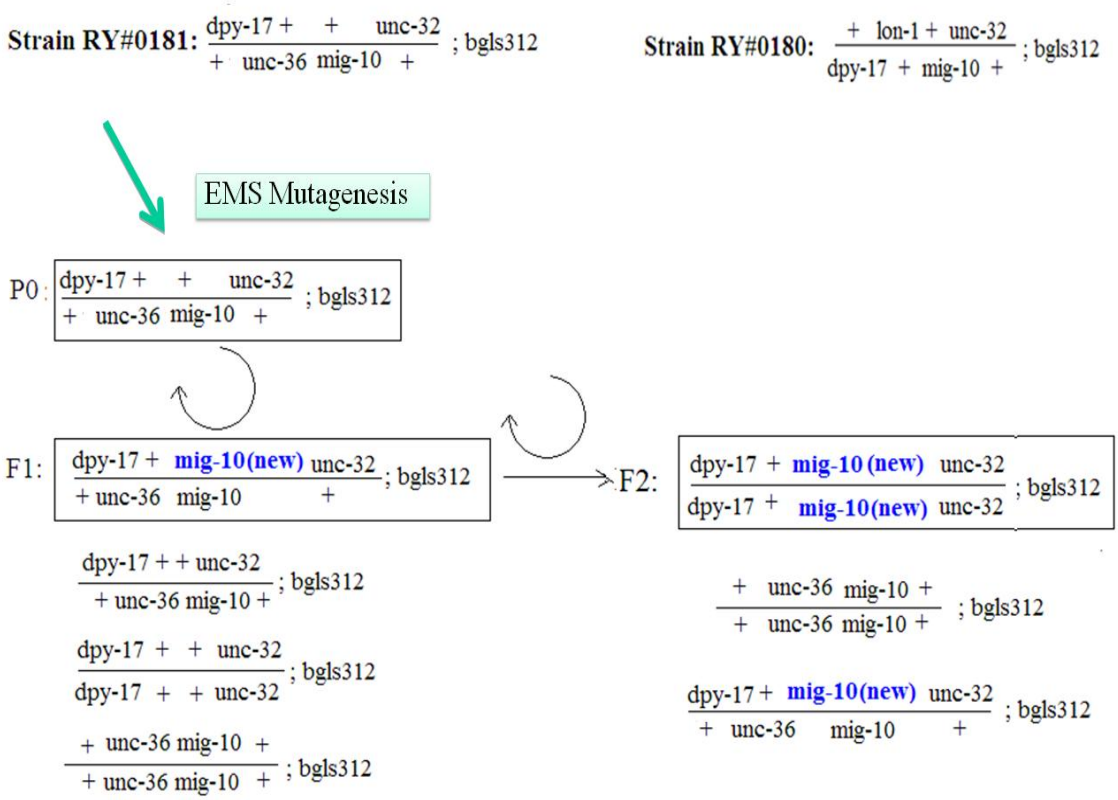

Figure 10: Screening Process for Strain RY0181

Again, F1 worms with the rare Mig phenotype were picked as putatives. True new mutants should produce an F2 generation with 25% worms with Dpy, Unc-32, Mig phenotype, 25% worms with Unc-36, Mig and 50% Mig. The phenotype that indicated a novel mutation was Dpy, Unc, Mig (DUM). The new *mig-10* mutations has been homozygosed in worms with the DUM phenotype, which makes the worm look short, fat

and coiled with a truncated excretory cell *(Figure 10).* However there was no DUM worms obtained in the eight screens using Strain RY0181.

3.6 Summary of Genetic Screens

Eight non-complementation screens were completed using the methods described previously (*Appendix B*). A total of 8360 genomes were screened in this project.
The number of haploid genome was calculated in each screen based on the number of heterozygous worms in F1 generation because these are the only worms that it is possible to see a new *mig-10* mutation in. For example if three mutagenized P0 worms produce about 100 progeny in the F1 generation, 50% of these progeny would be heterozygous. Therefore, 50 haploid genomes would be screened. The number of genomes screened in each of the eight screens completed can be seen in Table 2.

Table 2: Summary of Genetic Screens

	Strain RY0180	Strain RY0181						
Screen Number	Screen 1	Screen 2	Screen 4	Screen 5	Screen 6	Screen 7	Screen 8	Screen 9
Number of F1 plate	32	16	24	15	24	15	24	18
Average Progeny in F1 plate	105	100	80	100	100	100	110	100
Haploid Genome Screened	1680	800	960	750	1200	750	1320	900
Total Haploid Genome	8360							
Number of Putatives in F1 generation	8	2	15	7	30	16	23	6
Total Number of Putatives	107							
Number of LUM or DUM in F2 generation	13	5	9	0	7	7	0	2
Total Number of LUM or DUM	43							

Through all the screens, 107 worms with the Mig phenotype were produced. Of these, 43 putative progeny were produced in the F2 generation *(Table 2)*. These worms all seemed to display the correct phenotype that would indicate that a novel mutation had been generated; however their progeny in F3 generation did not also display the correct phenotype. Thus, the putatives were likely due to crossing over or identification errors.

4 Discussion

4.1 Non-complementation screening of RY0180 and RY0181

The original goals of this project were to find and characterize new mutations in the *mig-10* gene of *C. elegans*. There are several different approaches that can be used to attempt this, however the approach chosen was mutagenesis and non-complementation genetic screening. This process allowed us to confirm that any new mutation that was isolated and characterized was in the *mig-10* gene. In addition this technique didn't require any additional step such as a male cross. This technique was also chosen for its use of the mutagen Ethane Methyl Sulfonate (EMS) which provided a known statistical value, about 1 mutation in 5000 haploid genomes, for getting a mutation that would induce single base pair changes that would be more likely to create the kind of missense mutation we were hoping for. Knowing the statistical probability is also important because of the known time constraint. The statistic gave the minimum number of genomes that needed to be screened during the course of the project to realistically isolate a novel mutation. The secondary objective, if a mutation was isolated and characterized, was to use that mutation to study the signal transduction pathway of the *mig-10* gene, and possibly the active domains of the protein.

4.1.1 Problems with Strains RY0180 and RY0181

Screens 1 and 2 were used to test which strain of *C. elegans*, RY0180 or RY0181 would be the best to use for this project. Strain RY0180, used in screen 1, had the unanticipated problems of crossing over events and an unexpected phenotype. The results of a new mutation in *mig-10* gene should have produced a chromosome with mutations in

the *lon-1*, *mig-10*, and *unc-32* genes, which would have produced the phenotype Lon, Unc, and Mig. However, although this phenotype can be identified, it is easily confused with another phenotype. The *mig-10* gene often produces another characteristic in addition to the truncated excretory canal, a "withered tail". The withered tail phenotype is characterized by part of the tail being thinner than the overall width of the body. This however has the side effect of an affected pattern of movement by the worm, making it appear Unc, especially when the worm is long. Additionally the combined effects of the "withered tail" problem and crossing over events in Strain RY0180 made the identification of new mutations more difficult. If there was crossing over between the *lon-1* gene and the *mig-10* gene among the mutagenized P0 generation, it would give rise to 25% worms with Lon, Mig phenotype in F2 generation. The Lon, Mig worms display the "withered tail" phenotype and this makes the worm look uncoordinated when they do not actually have a mutation in the *unc-32* gene. This phenotype caused the worms to look like the putative LUM worms that indicate a novel mutation and led to many false positives.

Screen 2 was performed on the Strain RY0181, which has the chromosome *dpy-17 unc-32,* and *unc-36 mig-10*. The uncoordinated phenotype of *unc-36* can be more difficult to distinguish than that of *unc-32*. However, crossing over is rare between *mig-10* and *unc-36*, due to the close proximity of the genes. Consequently, it was decided that all subsequent screens would continue using the RY0181 strain.

4.1.3 General Problems in Non-complementation Genetic Screens

Out of the screens and the 43 possible putative progeny picked in F2 generation, we were unable to identify a new mutation. There are several possible explanations for this. A possibility is the high mortality rate and low birth rate of the animals, which proved that

mutagenesis was occurring but lowered the chances of finding a mutation. The general trend was a lower number of progeny than expected per plate. It is possible that a worm which did have a new mutation in the *mig-10* gene was unable to reproduce or died before reproducing. Another explanation is that there were a high number of false positives, due to the variability of the phenotypes, especially in strain RY0181. Worms which may have appeared to be Mig in the F1 generation were not, this can happen for different reasons such as the distortion of the excretory canal due to the age of the worm. Additionally the Unc phenotype varies and there can be a great range in the severity of the phenotype. For strain RY0181 which carries the gene *unc-36,* in the case of a less severe phenotype, the movement may appear to be normal. If the movement appears normal, it can be mistaken for a worm with only the *mig-10* genotype instead of the actual genotype of *unc-36 mig-10*.

4.2 Future Screening Possibilities

Even though we were unable to isolate a novel mutation, the information gained on refining this process can be used to assist future work in this area. By identifying the areas of the screen which can have problems, and finding solutions, future work done in this area can avoid these issues. This would enable the project to resume as opposed to being restarted.

Throughout the course of this project several parts of the method were refined. The first was to learn to recognize the behavioral patterns and phenotypes of each type of worm that may be encountered. With pre-constructed strains this is only a small number of phenotypes. These should be learned and well understood before screening begins in order to reduce the number of false putatives picked and the number or possible putatives missed. Another issue that arose was the high mortality rate of the mutagenized worms. To offset

this problem a larger number of worms should be mutagenized per screen and the worms must be at the correct stage of development. A schedule for maintaining the worm strains was also worked out. If the worms are kept at 15^{o}C they can be maintained every four days or so depending on the thickness of the bacterial lawn. A good process of troubleshooting was also developed. When troubleshooting was necessary it was important to look at every step and every ingredient to make sure they were done and made correctly. Some problems we encountered that required these troubleshooting skills were in making plates and running the gel after PCR.

4.2.1 Simple Screen

Several other aspects should be considered before the project resumes. Non-complementation screening has several advantages, but it also has some obvious disadvantages; the most obvious is that in this screen it is very easy to overlook a potential putative. By switching to a simple F2 screen, the probability of overlooking the correct putative is greatly decreased because the animals which exhibit a Mig phenotype are already homozygous for the new mutation.

The use of the simple screen would change the focus from screening to PCR, gel electrophoresis, and genetic crossing, because these techniques will be used to distinguish between the new mutations in the *mig-10* gene and any other mutations that result in the Mig phenotype. To perform a simple screen, a strain that is wild type except for the bgIs312 transgene would be mutagenized. F2 offspring would be screened for a truncated excretory canal. Mutant F2 progeny would be crossed with animals homozygous for the old mutation in *mig-10*. If all cross-progeny displayed the Mig phenotype, this would confirm that the new mutation is in the *mig-10* gene. Then the results could be processed using PCR

and gel electrophoresis. This is possible because the known mutation in the *mig-10* gene eliminates a restriction site; it produces a distinct restriction pattern, three bands, different from the pattern of the wild-type gene, four bands. New mutations can be compared to the two restriction maps to determine if it is a novel mutation and then sequenced to determine the exact sequence of the mutation.

4.2.2 Male Crossing Genetic Screen

The other technique to be considered is utilizing male crossing. Male crossing involves crossing a wild-type (N2) male with a wild-type hermaphrodite homozygous for the transgene bgIs312

(

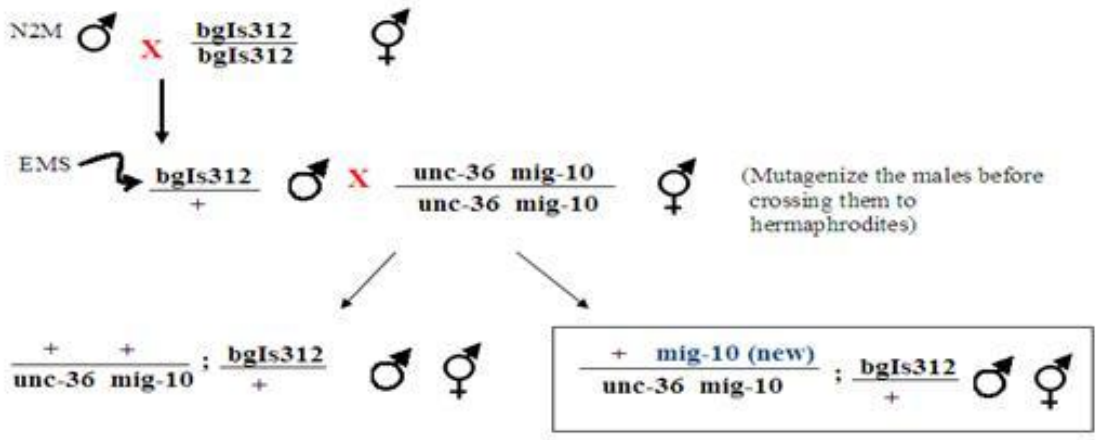

Figure 11*).* The males that are now marked with bgIs312 are mutagenized. This male worm would cross with a hermaphrodite that is homozygous for *unc-36* and *mig-10*. The progeny with the Mig phenotype are then isolated. *The mig-10* phenotype is the result of a mutation in the male *mig-10* gene. Self fertilization of the hermaphrodite would result in a phenotype of Unc and Mig, and a cross with a wild-type or *mig-10* phenotype from the male would result in worms with a wild-type phenotype or a Mig phenotype respectively

(

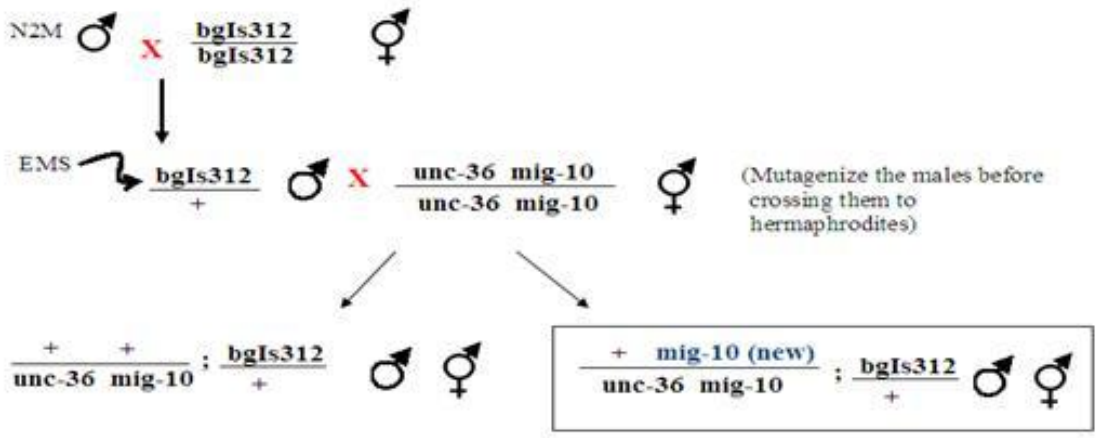

Figure 11*)*. In order to find worms homozygous for the new mutation in the *mig-10* gene, the next generation should be screened for worms with the Mig phenotype.

One big advantage of this screen is that only the cross progeny would be screened, because only they would contain the *bgIs312* transgene *(Figure 11)*. Thus, it would not be necessary in the initial screen to distinguish animals mutant for *mig-10* from those mutant for both *mig-10* and *unc-36*, which was a problem in screen 2 of this project. There a couple of problems associated with male crossing genetic screen, which include maintaining males, getting the mutagenized males to initially cross, and issues with getting the *bgIs312* marker into hermaphrodite cross-progeny.

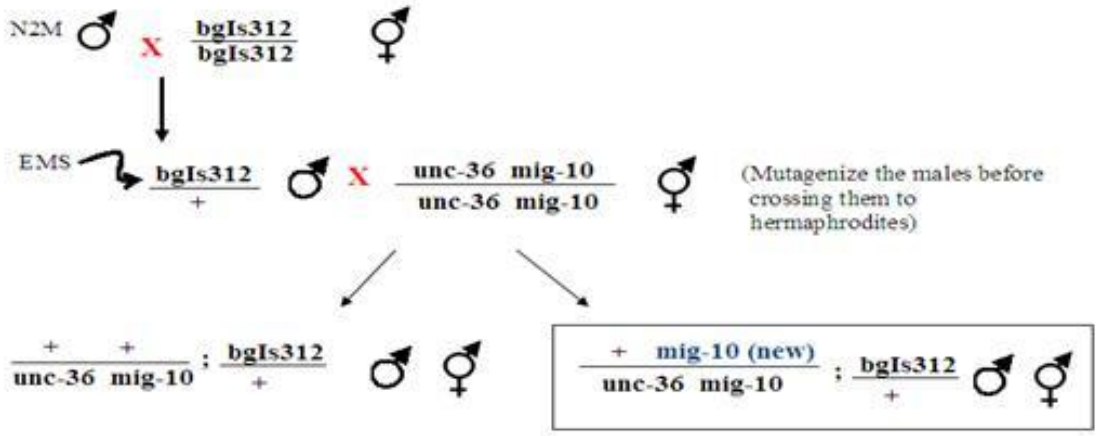

Figure 11: Process for Male Crossing

In conclusion, the most likely reason that we were unable to isolate a novel mutation is the time restriction posed by the school year. This project should be resumed because further experimentation should lead to a new mutation. We would recommend continuing to use the RY0181 strain if the project continues using the non-complementation screen. However we would advocate switching to the simple screen because of the time needed to learn to recognize specific phenotypes such as Unc-36 that is required to do the non-complementation screen, as well as the decrease in the probability of missing a mutation.

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Appendix A: Protocols

EMS Mutagenesis

WARNING: Be VERY careful handling EMS. It is a potent carcinogen! Wear gloves! EMS must be kept in the fume hood. After you add it to your worms, cap the tube and cover the tube with parafilm for additional security. Be very careful removing washes after the mutagenesis. Pipette all washes onto NaOH pellets (in a 50 ml screw cap tube) to neutralize the mutagen. After this treatment, you can pour the solution down the drain.

1. 3 days before mutagenesis:

   Grow up strain to mutagenize. (Pick 5 dishes of 3 hermaphrodites/dish of the strain.)

2. Day of mutagenesis:
   - α) Make sure there are lots of L4s/young adults on the plates you grew up. Don't bother continuing if worms are too young (wait till tomorrow) or too old (start over). (L4s/young adults will be generating lots of the gametes that you want to mutagenize.)
   - β) Fill a 50 ml polypro tube to about the 5 ml mark with NaOH pellets; set aside (for disposal of EMS).
   - χ) Wash worms off plates to a 15 ml tube using M9. (Pipette about 1 ml of M9 to a plate; shake plate back and forth to dislodge worms; use Pasteur pipette to transfer worms and M9 to a 15 ml polypro screw-cap tube).
   - δ) Wash worms 1-2X if many worms are still on plate.
   - ε) Spin worms down in table-top centrifuge for 2 minutes at 200xg
   - ϕ) Remove supernatant and then add 10 ml M9 to the tube, afterward invert tube several times to mix.
   - γ) Spin worms down again, remove supernatant, and resuspend worms in 2 ml M9.
   - η) Set up materials needed for the EMS mutagenesis under the hood:
     - parafilm

- tape and marker (labeling "mutagenesis" section to cautious people when approaching the hood)
- a tube containing 2mL M9
- worm tube
- several clean Pasteur pipette tips in a 50mL tube
- gloves and goggles

ι) Add 2 ml of EMS solution to prepared tube containing 2mL M9 and then add the EMS solution to worm tube. **Be CAREFUL! Wear gloves!** (Put pipette tips that touch EMS in your 50 ml tube of NaOH pellets to dispose of later. Worms will now be in 4 ml total volume)

φ) Rotate 4 hours at 20^{o} C.

κ) Spin down worms as before. Wash worms 3-4X with M9. **Be CAREFUL! Wear gloves!** These supernatants contain EMS! Pipette the supernatants carefully to your tube of NaOH pellets. (After your washes, the amount of EMS remaining will be miniscule; you don't need to wear gloves after this step.)

λ) Spin down worms. Resuspend in small volume (2-3 drops/dish).

μ) Pipette worms to 2-3 new dishes. Pipette worms to area away from food, so that they have to crawl to the food.

ν) After 1 hour, pick three or four L4 hermaphrodites or young adults (vulva, no eggs). (Pick animals that were healthy enough to crawl to the food; mutagenesis can kill worms, and you want the ones that will happily produce lots of progeny.)

ο) Incubate O/N at 20^{o} C.

3. Day after mutagenesis:
   Distribute gravid adults (animals with eggs inside) 3 or 4 worms to corresponding new plates.

Making NGM Agar Plates (Short Version)

Pour the Plates:

1. For 1 Liter of NGM Plates:
   - 3g NaCl
   - 17g Bacto-Agar
   - 2.5g Peptone
   - 975mL DI water
2. Autoclave the media for 30minutes. With the agar, you also need to autoclave:
3. After the autoclave run is finished, put the flask on a stir plate to stir and cool for one hour.
4. During the hour that the agar is cooling, clean the hood with 70% ethanol. Carefully, without allowing the tops to become separated from the plate bottoms, remove the plates from their sleeves and set up in stacks of 5 or 6.
5. When the flask has cooled, use sterile techniques to add:
   - 1mL 1M $CaCl_2$
   - 1mL 0.5% Cholesterol
   - 25mL 1M 6.0pH KPO_4
   - 1mL 1M $MgSO_4$
6. Pour 10mL per plate, let dry for 2-3 days.
7. Make overnight culture of OP50 in LB Broth.
8. Spot plates with about 100µL (3 drops with Pasteur pipette) after drying plates, let bacteria grow for 2 days before using.

Recipes for plate stocks

1M $CaCl_2$: 11g $CaCl_2$/100mL DI water, autoclave

0.5% Cholesterol: 500mg cholesterol/100mL Ethanol

1M 6.0pH KPO_4: 108.3g KH_2PO_4, 35.6g K_2HPO_4/ 1L DI water, test PH, autoclave

1M $MgSO_4$: 24.6g/100mL DI water, autoclave

LB Broth: 10g Tryptone, 5g Yeast, 10g NaCl, 1L DI water, autoclave

10 Worm PCR

Lysis Step

Proteinase K stock

- 10 mg/ml proteinase K (Boehringer-Mannheim) in water
- Make 10uL aliquots and store them at –20^{o}C

0.5mL Lysis buffer

- 50 mM KCL
- 10 mM Tris (pH 8.3)
- 2.5 mM $MgCl_2$
- 0.45% Nonidet P-40 (NP-40)
- 0.45% Tween 20
- 0.01% (weight/volume) gelatin
- Autoclave and store in aliquots (say, 0.5 ml aliquots) at –20^{o}C

1. Just before use, add 6uL of 10mg/mL proteinase K to 0.5mL lysis buffer.
2. Transfer a single worm (or more, for 10 worm version) using platinum wire 'worm picker' from the culture plate to a 2.5 uL drop of lysis buffer in the cap of a 0.2 or 0.5 ml tube suitable for thermo cycling (tube still attached to cap). Try not to transfer lots of bacteria (pick worms that are not in bacteria).
3. Make sure worms actually got into drop.
4. Close tube, centrifuge briefly. Add drop of mineral oil. Freeze sample at –70^{o}C for 10 minutes. Samples can be stored at –70^{o}C for weeks.
5. When ready to process samples, heat tube to 60^{o}C for 1 hour, followed by 95^{o}C for 15 minutes. Then keep sample at 4^{o}C until PCR reaction mix is added.

10 Worm Short PCR

Recipe for PCR

- 2.5uL 2.5mM dNTP mix
- 2.5uL 10X Long Template PCR System Buffer 3 (Roche Brand)
- 0.75uL 5 Units Taq polymerase
- 2.5uL 3uM primer 1 (*mig-10* WT1)

- 2.5uL 3uM primer 2 (*mig-10* WT2)
- 0.75uL Taq polymerase (5U/uL)
- 11.75uL dH_2O

Total: **22.5uL** master mix per sample

1. Vortex the master mix after making it and then spin it down.
2. Add 22.5uL of master mix to each worm sample.
3. Flick the tubes to mix well. Spin down.
4. Transfer tubes immediately to thermal cycler. Cycle 30 times with following conditions:
    i) 94°C for 30 seconds
    ii) 58°C for 1 minute
    iii) 72°C for 1 minute

Note: PCR reactions should always include a positive control and a negative control.

Digestion Reaction

1. Receipt for each digestion mix is listed as followed:
    - 10uL amplified PCR samples from each tube
    - 1.5uL 10X NEBuffer 4
    - 1uL Hpy188I restriction enzyme
    - 2.5uL dH_2O

    Total: **15uL reaction mix in each tube**

2. Incubate each digestion tube at 37 degree C overnight

Gel Electrophoresis

1. Prepare 1% agarose gel: weigh out 0.5g of agarose into a 25mL conical flask. Add 50mL of 1X TBE, swirl to mix. Note: it is good to use a large container, as long as it fits in the microwave, because the agarose boils over easily.
2. Microwave for about 1 minute to dissolve the agarose
3. Leave it to cool on the bench for 5 minutes down to about 60 degree C.
4. Pour the gel slowly into the tank. Insert the comb and double check that it is correctly positioned. Take out the comb after gel is formed.

5. Pour 1X TBE buffer into the gel tank to submerge the gel to 2-5mm depth.
6. Transfer 10mL of each sample from digested tubes and undigested tubes to a fresh microfuge tube.
7. Add 2uL 6X loading buffer and 1uL 10X SYBG Green buffer to each sample.
8. Load the first well with 4uL marker which include 0.8uL 6X loading buffer and 0.4uL SYBG green buffer.
9. Continue loading the samples and finish off with a final lane of marker.
10. Close the gel tank and run the gel at 100-150Volts for an hour or two hours.

Appendix B: Data of Genetic Screens

Table 3: Data for Genetic Screen 1, Strain RY0180

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected LUM were picked	Comment
39Z-1	100	1	1	50	3	
39Z-2	100	0	N/A	N/A	N/A	
39Z-3	100	3	1	50	2	
39A-1	100	0	N/A	N/A	N/A	
39A-2	100	0	N/A	N/A	N/A	
39A-3	100	2	0	N/A	N/A	
39A-4	100	0	N/A	N/A	N/A	
39A-5	100	0	N/A			
39A-6	100	1	0	N/A	N/A	
39A-7	100	0	N/A	N/A	N/A	
39A-8	100	1	1	50	4	
39A-9	100	1	0	N/A	N/A	
39A-10	100	1	0	N/A	N/A	
39A-11	100	2	0	N/A	N/A	
39A-12	100	2	0	N/A	N/A	
39B-1	100	1	0	N/A	N/A	
39B-2	100	0	N/A	N/A	N/A	
39B-3	100	1	1	30	1	
39B-4	100	2	1	50	0	
39B-5	100	2	0	N/A	N/A	
39B-6	100	1	0	N/A	N/A	
39B-7	100	2	1	50	1	
39B-8	100	0	N/A	N/A	N/A	
39B-9	100	2	0	N/A	N/A	
39B-10	100	1	0	N/A	N/A	
39C-1	100	6	0	N/A	N/A	
39C-2	100	2	1	50	1	
39C-3	100	4	0	N/A	N/A	
39C-4	100	0	N/A	N/A	N/A	
39C-5	100	0	N/A	N/A	N/A	
39C-6	100	1	0	N/A	N/A	
39C-7	100	2	1	30	1	

***** 5 plates containing about 100 progeny were used in the mutagenesis

Table 4: Data for Genetic Screen 2, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
47A-1	100	0	N/A	N/A	N/A	
47A-2	100	0	N/A	N/A	N/A	
47A-3	100	2	0	N/A	N/A	
47A-4	100	2	0	N/A	N/A	
47A-5	100	2	0	N/A	N/A	
47A-6	100	0	N/A	N/A	N/A	
47A-7	100	0	N/A	N/A	N/A	
47A-8	100	2	0	N/A	N/A	
47A-9	100	2	1	50	5	
47A-10	100	0	N/A	N/A	N/A	
47A-11	100	2	1	50	0	
47A-12	130	2	0	N/A	N/A	
47A-13	115	0	N/A	N/A	N/A	
47A-14	100	1	0	N/A	N/A	
47A-15	100	3	0	N/A	N/A	
47A-16	130	1	0	N/A	N/A	

Table 5: Data for Genetic Screen 5, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
5P0.1	75	0	0	N/A	N/A	
5P0.2	130	1	0	N/A	N/A	
5P0.3	130	2	0	N/A	N/A	
5P0.4	90	1	0	N/A	N/A	
5P0.5	50	1	0	N/A	N/A	
5P1.1	130	5	2	40	0	
5P1.2	100	2	1	40	0	
5P1.3	150	2	1	40	0	
5P1.4	15	0	N/A	N/A	N/A	
5P1.5	75	1	0	N/A	N/A	
5P2.1	150	0	N/A	N/A	N/A	
5P2.2	150	0	N/A	N/A	N/A	
5P2.3	100	0	N/A	N/A	N/A	
5P2.4	30	1	1	30	0	
5P2.5	75	3	2	30	0	

Table 6: Data for Genetic Screen 4, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
4P0.1	100	1	1	30	0	
4P0.2	100	1	1	30	0	
4P0.3	100	2	1	50	1	progeny were not Mig, but Dpy, Unc
4P0.4	50	0	N/A	N/A	N/A	
4P0.5	No Progeny	N/A	N/A	N/A	N/A	
4P0.6	50	1	1	30	0	
4P0.7	50	0	N/A	N/A	N/A	
4P0.8	50	0	N/A	N/A	N/A	
4P1.1	100	2	1	50	1	Progeny were not Mig, but Dpy, Unc
4P1.2	100	2	0	N/A	N/A	
4P1.3	50	0	N/A	N/A	N/A	
4P1.4	70	0	N/A	N/A	N/A	
4P1.5	50	0	N/A	N/A	N/A	
4P1.6	50	0	N/A	N/A	N/A	
4P1.7	70	0	N/A	N/A	N/A	
4P1.8	100	1	1	30	0	
4P2.1	100	1	1	50	0	
4P2.2	100	1	0	N/A	N/A	
4P2.3	100	1	1	70	3	4P2.3A1 and 4P2.3A2: Progeny were not Mig, but Dpy, Unc; 4P2.3A3: Progeny were not Dpy, but Unc, Mig.
4P2.4	100	2	2	85	2	Progeny were not Dpy, but Unc, Mig
4P2.5	120	3	2	100	0	NO DUM
4P2.6	100	2	1	40	1	No Progeny
4P2.7	100	1	0	N/A	N/A	
4P2.8	100	2	2	50	1	Progeny were not Dpy, but Unc, Mig

Table 7: Data for Genetic Screen 6, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
6P0.1	120	4	3	30	2	Some of the progeny were Dpy, Unc; some progeny look wild-type
6P0.2	0	0	N/A	N/A	N/A	
6P0.3	100	5	5	30	1	The progeny were Dpy, Unc
6P0.4	100	4	2	20	0	
6P0.5	100	2	1	20	0	
6P0.6	75	2	2	50	3	The progeny were Dpy, Unc
6P0.7	75	3	0	N/A	N/A	
6P0.8	150	3	1	40	0	
6P1.1	130	1	1	30	0	
6P1.2	100	2	2	20	0	
6P1.3	100	1	1	30	0	
6P1.4	70	0	N/A	N/A	N/A	
6P1.5	70	0	N/A	N/A	N/A	
6P1.6	150	3	2	30	1	No Progeny
6P1.7	130	3	3	30	0	
6P1.8	100	1	1	30	0	
6P2.1	150	1	1	30	0	
6P2.2	50	0	N/A	N/A	N/A	
6P2.3	80	1	1	30	0	
6P2.4	120	0	N/A	N/A	N/A	
6P2.5	110	1	1	30	0	
6P2.6	135	2	2	30	0	
6P2.7	140	2	1	30	0	
6P2.8	100	0	N/A	N/A	N/A	

Table 8: Data for Genetic Screen 7, Strain RY0181

Mutagenized P0				F1	F2	
Mutagenized P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
7P0.1	125	5	3	50	2	
7P0.2	140	2	2	30	0	
7P0.3	150	2	1	30	0	
7P0.4	175	1	1	50	1	
7P0.5	115	2	1	50	1	
7P1.1	0	N/A	N/A	N/A	N/A	
7P1.2	150	3	2	30	0	
7P1.3	100	1	1	30	0	
7P1.4	100	3	3	30	3	
7P1.5	100	2	2	40	0	
7P2.1	0	N/A	N/A	N/A	N/A	
7P2.2	75	1	0	N/A	N/A	
7P2.3	15	0	N/A	N/A	N/A	
7P2.4	70	0	N/A	N/A	N/A	
7P2.5	10	1	0	N/A	N/A	

Table 9: Data for Genetic Screen 8, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
8P0.1	20	1	1	20	0	
8P0.2	20	0	N/A	N/A	N/A	
8P0.3	50	2	0	N/A	N/A	
8P0.4	No Progeny	N/A	N/A	N/A	N/A	
8P0.5	100	3	3	20	0	
8P0.6	100	1	1	20	0	
8P0.7	20	0	N/A	N/A	N/A	
8P0.8	20	0	N/A	N/A	N/A	
8P0.9	70	1	1	20	0	
8P0.10	50	0	N/A	N/A	N/A	
8P0.11	70	1	0	N/A	N/A	
8P0.12	50	0	N/A	N/A	N/A	
8P0.13	50	2	2	40	0	
8P1.1	30	1	1	30	0	
8P1.2	130	2		No Progeny		
8P1.3	120	3	3	20	0	
8P1.4	120	1	1	20	0	
8P1.5	120	2	2	20	0	
8P1.6	No Progeny	N/A	N/A	N/A	N/A	
8P1.7	100	0	N/A	N/A	N/A	
8P1.8	65	2	1	20	0	
8P1.9	80	2	2	20	0	
8P1.10	110	1	1	40	0	
8P1.11	85	3	2	40	0	
8P1.12	70	3		No Progeny		
8P1.13	55	2		No Progeny		
8P2.1	40	0	N/A	N/A	N/A	
8P2.2	20	1	1	40	0	
8P2.3	100	0	N/A	N/A	N/A	
8P2.4	60	0	N/A	N/A	N/A	
8P2.5	85	0	N/A	N/A	N/A	
8P2.6	70	0	N/A	N/A	N/A	
8P2.7	30	0	N/A	N/A	N/A	
8P2.8	70	0	N/A	N/A	N/A	
8P2.9	60	0	N/A	N/A	N/A	
8P2.10	110	0	N/A	N/A	N/A	
8P2.11	90	1	1	40	0	
8P2.12	85	0	N/A	N/A	N/A	
8P2.13	65	0	N/A	N/A	N/A	

Table 10: Data for Genetic Screen 9, Strain RY0181

Mutagenized P0				F1	F2	
P0 Plates	Progeny on Mutagenized P0 plates	Number of Putatives were picked	Number of putatives were Mig	Average Number of Progeny from Mig	Number of suspected DUM were picked	Comment
9P0.1	50	0	N/A	N/A	N/A	
9P0.2	50	0	N/A	N/A	N/A	
9P0.3	100	3	1	50	0	No DUM
9P0.4	120	2	1	70	2	suspected DUM were actually DM
9P0.5	50	0	N/A	N/A	N/A	
9P0.6	110	4	1	70	0	No DUM
9P1.1	30	0	N/A	N/A	N/A	
9P1.2	100	0	N/A	N/A	N/A	
9P1.3	80	1	0	N/A	N/A	
9P1.4	80	1	0	N/A	N/A	
9P1.5	100	1	0	N/A	N/A	
9P1.6	70	3	2	100	0	No DUM
9P2.1	120	3	0	N/A	N/A	
9P2.2	80	0	N/A	N/A	N/A	
9P2.3	100	0	N/A	N/A	N/A	
9P2.4	130	2	0	N/A	N/A	
9P2.5	30	0	N/A	N/A	N/A	
9P2.6	110	1	1	50	0	

Appendix C: The Map of *Mig-10* locus